Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

PNEUMOCOCCUS ANAPHYLAXIS AND IMMUNITY.*

E. C. ROSENOW.

(*From the Memorial Institute for Infectious Diseases, Chicago.*)

In the following pages I wish to report briefly the results of a study of anaphylaxis to pneumococcus protein and its relation to pneumococcus infection and immunity, as well as of a study of the increased reactivity of guinea-pigs to pneumococcus products and living pneumococci, as measured by the leukocytic count; and then to give a summary of a study of the effects of primary injection of certain pneumococcus extracts in NaCl solution, and a comparison of these with the symptoms produced by the injection of products obtained by the interaction of pneumococci, immune serum, and normal serum.

PNEUMOCOCCUS ANAPHYLAXIS IN SENSITIZED GUINEA-PIGS.

The view that anaphylactic intoxication in sensitized animals is due to rapid parenteral digestion of protein into toxic-cleavage products is supported strongly by recent investigations. The work of Vaughan[1] and his coworkers, of Abderhalden,[2] of Pfeiffer and Mita,[3] of Wells,[4] and others point strongly in this direction.

The protocols of the experiments on the production of active anaphylaxis are omitted, because the usual procedure, which is simple and well understood, was followed.

From a study of a long series of extracts, the following facts seem to be established: Guinea-pigs injected with dead pneumococci or pneumococcus extracts become hypersensitive to subsequent injection after a period of from eight to 12 days. It is quite immaterial whether the first injection is made subcutaneously, intravenously, intraperitoneally, or intrapleurally. The second

---

* Received for publication July 17, 1911.

[1] *Ztschr. f. Immunitätsf. u. exp. Therap.*, 1911, 9, p. 458.

[2] *Ztschr. f. physiol. Chemie*, 1910, 64, p. 423.

[3] *Ztschr. f. Immunitätsf. u. exp. Therap.*, 1910, 4, p. 410.

[4] *Jour. Infect. Dis.*, 1909, 6, p. 506.

dose of the clear pneumococcus extract, however, must be injected into the heart or intravenously in order to produce fatal anaphylactic shock. The symptoms produced are irritability, dyspnea, convulsions, abrupt drop in temperature and leukocytes, urination, and at times defecation. After death the lungs show the usual pallor and emphysema.

The minimum sensitizing dose of a clear pneumococcus extract in NaCl solution for guinea-pigs weighing 250 gms. is approximately 0.01 c.c. Smaller doses do not sensitize regularly. From four to seven c.c. of clear, filtered extract in salt solution, if prepared at 37° C. for 24 hours or in the ice chest for a longer period, is sufficient to cause fatal shock regularly. The injection into the left heart causes more marked symptoms than injection into the right heart, or into the jugular vein. Sensitization has been found to last as long as 410 days. The mother sensitized before or early in pregnancy transmits sensitiveness to the first litter but not to the second litter. Fresh suspensions of living pneumococci in NaCl solution do not cause fatal shock in sensitized pigs. It is necessary first to bring into solution a certain proportion of the pneumococci. The serum of sensitized guinea-pigs, when injected into a normal pig, makes the latter sensitive at the end of 12 to 24 hours (passive anaphylaxis). After severe and fatal anaphylactic shock, the serum is not toxic to normal guinea-pigs, nor does it sensitize to subsequent injection of pneumococcus extracts. Boiling pneumococci in NaCl solution and then extracting them does not interfere materially with the production of shock in sensitized pigs, nor does it destroy the sensitizing principle.

The pneumococci used in the preparation of extracts were obtained from the blood of pneumonia patients and were grown chiefly in ascites broth, broth, and on blood agar. The extracts were prepared at first by suspending approximately six to 10 billion pneumococci per c.c. of NaCl solution; later the suspensions were made so that one c.c. of the extract represented the soluble substances in approximately two to five billion cocci.

The effect of injection of pneumococcus protein brought into solution by various methods has been tested. The extracts prepared by repeatedly freezing and thawing the suspensions; prolonged suspension in NaCl solution in the ice chest; and those prepared at 37° C. for 24 hours or for a longer period, if non-virulent pneumococci are used, cause no symptoms on primary injection, but always in sensitized animals.

The extracts prepared from highly virulent pneumococci at 37° C. for 24 hours or longer, on the other hand, sensitize but do not always produce anaphylactic shock in sensitized pigs, and are found at times to produce symptoms in normal pigs which cannot be differentiated from typical anaphylactic shock. The extracts prepared in NaCl solution under ether at 37° C. for 48 hours and to a lesser degree those prepared over chloroform are especially apt to give primary symptoms (see Table 4). Morphologically, the pneumococci in the former methods are fairly well preserved and their affinity for basic dyes is only moderately diminished. They retain Gram's stain. In the latter, they are much disintegrated, stain faintly by basic dyes, are Gram negative, and the affinity for acid dyes is greatly increased. Extracts of pneumococci obtained from various sources, cultivated for various periods (one strain for nine years), and all grades of virulence sensitize and intoxicate with respect to each other when given in proper doses. It makes no difference whether they are cultivated in broth or on agar, whether washed or unwashed, before the extract in NaCl solution is made.

It was hoped that in the anaphylactic reaction we might find an additional test for differentiating pneumococci from *Streptococcus pyogenes*, but the experiments show that streptococcus extracts sensitize with respect to pneumococcus extracts and vice versa, while injection of streptococcus extracts rarely produce fatal shock, whether the animal is sensitized with either streptococcus or pneumococcus extracts. The symptoms are usually more marked in pigs sensitized with streptococci, and later injected with streptococcus extracts, but the difference is not great enough to be reliable. Staphylococcus extracts do not sensitize with respect to pneumococcus extracts or vice versa. Extracts of *Streptococcus mucosus* behave exactly as do those of highly virulent pneumococci.

The hypersensitive state of guinea-pigs to pneumococcus extracts disappears after intravascular injection of a large, but not fatal, dose of pneumococcus extract, after repeated intraperitoneal or subcutaneous injections of extracts or dead pneumococci, and after recovery from a pneumococcus infection.

The question of anaphylaxis to pneumococcus extracts in relation to susceptibility and immunity to pneumococcus infection has also been studied. It has been found from numerous tests that guinea-pigs which are sensitive to pneumococcus extracts resist small injections of virulent pneumococci (from 0.01 to 0.2 c.c. of 24-hour broth culture, depending on the virulence), but are more susceptible to large injections than the normal controls. The peritoneal smears made after inoculation, and smears and plate cultures made from the blood after death bring out the interesting fact that disintegration of pneumococci (as shown by the capsular stain I have devised) in the sensitized pig is greater and the number of viable cocci in the blood after death is far less than in normal pigs. This resistance offered by the blood stream to pneumococci, from the evidence at hand, would seem to be best explained on the ground of a rapid disintegration of cocci. The promptness with which pneumococcus extracts, when autolysis is prevented, are split into toxic components in sensitized pigs, is in keeping with this idea.

Moreover, the intraperitoneal injection of a properly gauged dose of virulent pneumococci into sensitized pigs is followed in two to three hours by a sharp leukocytosis not observed in normal pigs, which succumb, while large doses produce an earlier leukopenia and death. The exudation of leukocytes into the peritoneal cavity is greater where leukocytosis is observed in the blood. The intimate relation of anaphylaxis to pneumococcus immunity is shown also by the more rapid disappearance (chiefly by phagocytosis) of non-virulent and autolyzed virulent pneumococci from the peritoneal cavity of sensitized pigs.

In other words, guinea-pigs which are hypersensitive to pneumococcus products are to a certain degree immune to pneumococcus infection, the early death of the sensitized pigs receiving the large dose being really an expression of heightened reactivity. From the study of this point, by comparing the amount of disintegration and the number of living pneumococci (as determined by the blood-agar plate method) in the peritoneal cavity of normal and sensitized pigs, it seems certain that the pneumococci in the latter not only disintegrate more rapidly, but also multiply more rapidly, so that the early death in the sensitized animal receiving a large dose is not due wholly to the rapid destruction of the injected cocci.

In connection with active pneumococcus anaphylaxis another point of great interest should be mentioned. It has been observed many times that guinea-pigs and, to a lesser degree, rabbits, are apt to die in from eight to 12 or 14 days following the injection of relatively large doses of heated pneumococcus extracts or heat-killed pneumococci. This rarely occurs when unheated extracts in which autolysis has been carried to a more extreme point are injected, or when autolyzed pneumococci are injected. In the latter it occasionally occurs, but only when huge doses (90 billion) are introduced. It makes no difference whether the injection is made subcutaneously, intraperitoneally, or intravascularly. The time of death corresponds quite closely to the time necessary to render pigs hypersensitive. This observation may now be explained on the ground that the pneumococcus protein lodges in the tissues until antibodies are formed, and then is rapidly split into toxic products which prove fatal. The observation, which I have made many times, that animals injected with only slightly virulent living pneumococci often seem perfectly well until after seven to 10 days, and then succumb to the infection, is in keeping with this idea. Clinical observations go to show that many toxic processes of bacterial infection may be explained on the basis of anaphylaxis or allergy, as emphasized especially by von Pirquet and Schick,[1] von Pirquet,[2] and Friedberger.[3] They assume that bacteria in themselves have little or no toxic properties, and that intoxication occurs first after interaction of bacterial protein (antigen), antibody, and complement (the two latter being contained in the serum of the host) has taken place.

---

[1] *Wien. klin. Wchnschr.*, 1903, 16, p. 758.

[2] *Arch. of Int. Med.*, 1911, 7, p. 275.

[3] *Deut. med. Wchnschr.*, 1911, 37, p. 481.

THE INCREASED REACTIVITY OF GUINEA-PIGS AFTER INJECTIONS OF AUTOLYZED PNEUMOCOCCI.

In studying the effect of autolyzed pneumococci on intraperitoneal injection in guinea-pigs it was noted that the primary injection is not followed by leukocytosis in the peripheral circulation. A relatively slight exudation of leukocytes takes place. A second injection given from eight days to four months later is followed by a prompt intravascular leukocytosis and a more rapid exudation of leukocytes. Disappearance of pneumococci is correspondingly more rapid and takes place largely by phagocytosis. In these experiments, unless otherwise indicated, autolysis was carried out in the usual way by suspending virulent pneumococci in two changes of NaCl solution, and keeping them at 37° C. for two periods of 48 hours each. Usually chloroform was added, but pneumococci autolyzed in NaCl solution without chloroform behave similarly in this respect. It makes no difference where the first injection is made. The second injection, however, must be made intraperitoneally, intrapleurally, or into the circulation to bring out the reaction sharply. The reaction is specific.

Table 1 shows that autolyzed pneumococci cause no increase in leukocytes when injected intraperitoneally (No. 98) in normal animals. This has been found true even when the dose was made as large as 90 billion pneumococci. A second injection in from nine days to as long as four months later of the same dose is followed by a prompt leukocytosis. Clear pneumococcus extracts in this way sensitize to subsequent injections of extract as well as to autolyzed pneumococci (Nos. 127 and 44). Pneumococci autolyzed in salt solution, and then digested in pneumonic serum at 37° C. for 24 hours to four days, and then washed in salt solution, still contain the substance which sensitizes for pneumococci autolyzed in NaCl solution, but do not contain enough of the substance which produces leukocytosis when injected a second time. The clear serum, after pneumococci have been suspended in it, also contains the sensitizing principle (Nos. 92, 163, 117, and 101), but not that which causes leukocytosis. Parallel tests of the effect of normal serum show that pneumonic serum is more active, disintegration of autolyzed pneumococci being more rapid. Pneumococci auto-

TABLE 1.

INCREASED REACTIVITY OF GUINEA-PIGS AS MANIFESTED BY LEUKOCYTOSIS FOLLOWING INJECTIONS OF AUTOLYZED PNEUMOCOCCI AND PNEUMOCOCCUS EXTRACTS.

No. of Animal	Date and Injections	Leukocyte Count		
		Just Before	2½ Hours After	24 Hours After
93	6–30–10: 500 million pneumococci autolyzed in NaCl sol. intraperitoneally	5,200	6,000	5,800
	7–11–10: Same dose intraperitoneally	11,400	21,400	16,000
44	6–16–10: 4 c.c. ext. 143*a* into heart	.....	.....	.....
	6–24–10: 500 million autolyzed pneumococci intraperitoneally	9,700	17,900	13,600
119	7–18–10: 500 million autolyzed pneumococci intraperitoneally after suspension in 2 c.c. pneumonic serum at 37° C. 24 hrs. and washed in NaCl sol.	.....	.....	.....
	7–28–10: 500 million autolyzed pneumococci treated as above intraperitoneally	5,200	6,700	8,000
92	6–30–10: 500 million autolyzed pneumococci intraperitoneally	.....	.....	.....
	7–11–10: 500 million autolyzed pneumococci after treatment in 2 c.c. pneumonic serum at 37° C. 24 hrs. and washed in NaCl. sol.; intraperitoneally	10,400	8,800	13,800
163	10–8–10: 2.5 c.c. pneumonic serum after 800 million pneumococci were suspended in it for 4 days at 37° C., into heart	.....	.....	.....
	10–24–10: 500 million autolyzed pneumococci intraperitoneally	11,200	17,200	9,800
117	7–18–10: 500 million pneumococci autolyzed after suspension in pneumonic serum at 37° C. 48 hrs., washed in NaCl sol.; intraperitoneally	8,700	8,200	8,400
	7–27–10: 500 million autolyzed pneumococci intraperitoneally	7,200	18,000	9,000
155	10–3–10: 2 c.c. ext. 159 intraperitoneally	.....	.....	.....
	10–17–10: 500 million pneumococci autolyzed at 37° C. for 48 hrs. in NaCl under ether; intraperitoneally	5,400	7,200	7,600
101	7–12–10: 1 c.c. pneumonic serum (3 days before crisis) after 500 million autolyzed pneumococci suspended at 37° C. 24 hrs.; intraperitoneally	9,300	9,900	9,400
	7–23–10: 500 million autolyzed pneumococci intraperitoneally	12,000	17,400	.....
112	7–15–10: 500 million autolyzed pneumococci intraperitoneally	.....	.....	.....
	7–28–10: 500 million formalin-killed pneumococci after suspension in normal serum at 37° C. 48 hrs.; intraperitoneally	10,300	16,000	11,300
127	7–28–10: 4 c.c. ext. 144 intraperitoneally, 37° C. 24 hrs. and then on ice	.....	.....	.....
	10–24–10: Same injection intraperitoneally	10,100	20,600	15,500

TABLE 1.—*Continued.*

No. of Animal	Date and Injections	Leukocyte Count		
		Just Before	2½ Hours After	24 Hours After.
77......	6–16–10: 500 million autolyzed pneumococci intraperitoneally	.....	.....	.....
	6–24–10: 500 million autolyzed pneumococci intraperitoneally	14,800	22,000	20,900
	7–28–10: 4 c.c. ext. 144 into left heart*	12,800	4,300	13,200
	8–18–10: 500 million autolyzed pneumococci intraperitoneally	9,400	8,200	8,900

* Marked symptoms of anaphylaxis.

lyzed in NaCl solution under ether 48 hours lose much of the part which causes leukocytosis (No. 155), the cocci here, like those after treatment in serum, being more disintegrated. Heat and formalin (2 per cent in NaCl solution) killed pneumococci do not produce leukocytosis either on first or second injection, while partially serum-digested, formalinized, and heat-killed pneumococci produce leukocytosis in sensitized pigs. The fact that well preserved dead pneumococci do not produce leukocytosis is probably not due to their relative insolubility or greater resistance to phagocytosis, but rather to a substance which they contain which depresses the leukocytic mechanism.

During the disintegration of pneumococci, whether by the action of their own proteolytic ferment in salt solution or by the digestive action of serum, there goes into solution early a toxic substance (pneumococcus-anaphylatoxin) which lowers the temperature and opsonic index, and when injected in proper dosage depresses the leukocytic mechanism. There remains in the partially digested cocci a substance which produces a sharp leukocytosis when injected into sensitive pigs, and an abrupt rise in opsonin in man. Finally, on further digestion, both these substances are destroyed. It would seem that the leukopenia in overwhelming pneumococcus infection, the hyper-leukocytosis in severe but more favorable infections, and the slight leukocytosis in the mild cases may be explained on the basis of these results.

The experiments on guinea-pigs (No. 77) show the usual leukocytosis on second injection of autolyzed pneumococci; a leukopenia and severe anaphylactic shock on intravascular injection of extract (prepared so that little digestion takes place); and an absence of leukocytosis on injection of autolyzed pneumococci following this reaction. In other words, the reactivity of guinea-pigs to pneumococcus extracts and autolyzed pneumococci as measured by leukocytosis runs a course quite similar to that of sensitized pigs as measured by the symptoms and temperature. Moreover, the results obtained by this method support the idea that anaphylactic symptoms in sensitized animals are due to a parenteral digestion of protein, because autolyzed pneumococci are digested and destroyed more rapidly in sensitized pigs when leukocytosis is produced. This fact is shown strikingly in No. 77, the pneumococci disappearing most rapidly after the second injection which was followed by an increase of leukocytes.

ANAPHYLACTIC INTOXICATION OF NORMAL GUINEA-PIGS WITH PRODUCTS OBTAINED BY THE INTERACTION OF PNEUMOCOCCI, IMMUNE SERUM, AND NORMAL SERUM.

Friedberger[1] and Friedeman[2] were the first to obtain a highly toxic substance capable of causing the symptoms of anaphylaxis by the interaction of albumen or bacteria (antigen), immune serum (amboceptor), and normal serum (complement). The former concludes that the symptoms in infectious diseases are due to "anaphylatoxin," a split product of the various bacterial proteins. The existence of a separate poison for various bacteria, he says, is not proven nor necessary to explain the facts, the difference in the character of the infection by the different species being due, not to differences in bacterial proteins, but to different modes of attack on part of the host.

Neufeld and Dold[3] produced primary anaphylaxis in guinea-pigs by treating pneumococci, typhoid bacilli, or cholera vibrio with the corresponding immune serum, and later with complement. They believe that bacteriolysis prevents the formation of anaphylatoxic substance, because pneumococci are well preserved when the mixture produces fatal intoxication, and because they were able to get intoxication with cholera vibrio only when bacteriolysis was prevented by placing the mixture on ice. They are not certain whether the bacteria furnish the mother substance, but conclude that the three components, antigen, amboceptor, and complement, are essential to produce primary anaphylaxis.

---

[1] *Deut. med. Wchnschr.*, 1911, 37, p. 481.

[2] *Ztschr. f. Immunitätsf. u. exper. Therap.*, 1909, 2, p. 591.

[3] *Berl. klin. Wchnschr.*, 1911, 48, p. 55.

More recently Wasserman and Keysser[1] conclude that anaphylatoxin is not necessarily a split product of the bacterial antigen because barium sulphate, kaolin, and other inert substances act as well as bacteria with amboceptor and complement. They believe that the amboceptor furnishes the mother substance of the anaphylatoxin, and name the poisonous substance "toxopeptid," assuming that it is an early product of the ferment action of complement on amboceptor and not on antigen. They conclude that the bacterial antigen or barium sulphate alters the surface tension and adsorption properties, so as to favor the digestion of amboceptor by complement. Friedberger[2] considers, however, that the horse serum used by them as amboceptor acted in reality as antigen, the guinea-pig serum containing both complement and amboceptor. They showed that their mixtures are harmless when first made, very toxic at a certain period, and again harmless on longer treatment at 37° C.

As shown by Table 2, symptoms of anaphylactic intoxication may be obtained in normal guinea-pigs with pneumococci treated in various ways:

1. By the action of pneumococci of immune pneumococcus serum, immune streptococcus serum, humanized rabbit serum, heated normal goat serum followed with fresh normal guinea-pig serum (Table 2, experiments 1–12).

2. By treating pneumococci in ether or soap for a short time, or in NaCl solution at 37° C. for a longer period, instead of immune serum, and then suspending them in guinea-pig complement (Table 2, experiments 18, 21, 25). Ether-treated and soaped pneumococci also yield the substance if treated in immune pneumococcus serum and then in complement (Table 2, experiments 17 and 20).

---

[1] *Folia Serologica*, 1911, 7, p. 243.

[2] *Ztschr. f. Immunitätsf. u. exp. Therap.*, 1911, 9, p. 234.

TABLE 2.

ANAPHYLACTIC INTOXICATION OF NORMAL GUINEA-PIGS PRODUCED BY PNEUMOCOCCI UNDER VARIOUS CONDITIONS.

EXPERIMENT	PNEUMOCOCCI	SERUM TREATMENT OF PNEUMOCOCCI		SYMPTOMS	APPEARANCE OF PNEUMOCOCCI AT TIME OF INJECTION
		Immune Sera	Complement		
1	Virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 3 min.	Mostly Gram positive
2	Non-virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 4 min.	Mostly Gram positive
3	Autolyzed pneumococci	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	No symptoms	Dissolved
4	Boiled virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Moderate symptoms	Largely Gram positive but a fair number were negative
5	Boiled virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 72 hrs.	Marked symptoms	Largely Gram positive but a fair number were negative
6	Virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumococcus immune serum at 37° C. 1 hr.	4 c.c. in ice chest 48 hrs.	Death in 2 min.	Largely Gram positive but a fair number were negative
7	Virulent pneumococci from 30 c.c. broth culture	1 c.c. streptococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 2 min.	Largely Gram positive but a fair number were negative
8	Virulent pneumococci from 30 c.c. broth culture	1 c.c. humanized rabbit serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 4 min.	Largely Gram positive but a fair number were negative
9	Virulent pneumococci from 30 c.c. broth culture	1 c.c. fresh normal rabbit serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	No symptoms. No drop in temperature	Largely Gram positive but a fair number were negative
10	Virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumonic serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Marked symptoms but recovered	Largely Gram positive but a fair number were negative

11	Virulent pneumococci from 30 c.c. broth culture	3 c.c. normal guinea-pig serum	4 c.c. in ice chest 48 hrs.	No symptoms	Largely Gram positive but a fair number were negative
12	Virulent pneumococci from 30 c.c. broth culture	1 c.c. normal goat serum free from complement at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Marked symptoms but recovered	Largely Gram positive but a fair number were negative
13	Virulent pneumococci from 30 c.c. broth culture	....	4 c.c. NaCl sol. in ice chest 48 hrs.	No symptoms	Largely Gram positive but a fair number were negative
14	....	....	4 c.c. normal guinea-pig serum in ice chest 48 hrs.	No symptoms	....
15	....	....	4 c.c. normal guinea-pig serum 37° C. 24 hrs., then in ice chest 24 hrs.	Severe symptoms. Drop of 5° F.	....
16	....	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs. and then at 37° C. 18 hrs.	Death in 4 min. from typical symptoms. Lungs emphysematous	....
17	Virulent pneumococci from 30 c.c. broth treated with ether for ½ hr.	1 c.c. pneumococcus immune serum for ½ hr. at 37° C.	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Death in 2 min. from typical symptoms. Lungs emphysematous	Mostly Gram negative
18	Virulent pneumococci from 30 c.c. broth treated with ether for 40 min. at 37° C.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Death in 2 min. from typical symptoms. Lungs emphysematous	Mostly Gram negative
19	Virulent pneumococci from 30 c.c. broth	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	No symptoms	Mostly Gram positive
20	Virulent pneumococci from 30 c.c. broth treated in 1–2,500 sod.-oleate at room temp. 1 hr.	1 c.c. pneumococcus immune serum in ice chest 24 hrs.	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Death in 3 min. Typical symptoms. Lungs emphysematous	Mostly Gram negative
21	Virulent pneumococci from 30 c.c. broth treated in 1–2,500 sod.-oleate room temp. for 1 hr.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Severe symptoms. Nearly dead in 10 min. Drop of 2.5° F.	Mostly Gram negative
22	Virulent pneumococci from 30 c.c. broth treated with 1 c.c. human bile at 37° C. for ½ hr.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	No symptoms	All Gram negative and practically all dissolved

TABLE 2—*Continued.*

EXPERIMENTS	PNEUMOCOCCI	SERUM TREATMENT OF PNEUMOCOCCI		SYMPTOMS	APPEARANCE OF PNEUMOCOCCI AT TIME OF INJECTION
		Immune Sera	Complement		
23	Virulent pneumococci from 30 c.c. broth treated in 1–1000 sod.-oleate room temp. for 1½ hrs.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	No symptoms	All Gram positive and practically all dissolved
24	Virulent pneumococci from 30 c.c. broth treated in 25 per cent phenol at 37° C. 1 hr.	1 c.c. pneumococcus immune serum in ice chest 20 hrs.	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Marked symptoms. Drop of 3.7° C. in 10 min.	All Gram positive and practically all dissolved
25	Virulent pneumococci from 30 c.c. broth treated in 1 c.c. NaCl at 37° C. 24 hrs.	....	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Death in 4 min. Typical symptoms. Lungs emphysematous	Mostly Gram negative but disintegration not marked
26	3 c.c. ext. in NaCl from pneumococci in 30 c.c. broth at 37° C. 48 hrs.	....	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Death in 2 min. Typical symptoms. Lungs em-emphysematous	....
27	3 c.c. NaCl	....	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	No symptoms	....
28	Virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum 37° C. for ½ hr.	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Death in 2 min.	Mostly Gram negative
29	Virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum 37° C. for ½ hr.	4 c.c. normal guinea-pig complement heated 58° C. for ½ hr.	Death in 3½ min.	Mostly Gram negative
30	Virulent pneumococci from 30 c.c. broth	1 c.c. pneumococci immune serum 37° C. for ½ hr.	4 c.c. normal guinea-pig complement heated 58° C. for 1½ hrs.	Severe symptoms but recovered	Mostly Gram negative

NOTE.—The pneumococci were treated in immune serum as indicated, the latter removed and the treated cocci suspended in normal guinea-pig serum (15° C.) for the time given. Control tests showed that it makes no difference whether the pneumococci are removed before injection or not. Injections of guinea-pig complement alone after being kept in the ice chest never produced symptoms in my hands. The Gram stain is a good index of the degree of disintegration at the time of injection. All injections in Table 2 were made into the jugular vein.

3. By suspending pneumococcus extracts (in which autolysis has not been carried far enough to produce intoxication) in guinea-pig complement at 15° C. 24 hours (Table 2, experiment 26).

Identical symptoms may be obtained with normal guinea-pig serum alone if kept at 37° C. for the proper length of time (Table 2, experiments 15 and 16).

The intoxicating substance obtained by the interaction of pneumococci, immune serum, and complement is thermolabile (Table 2, experiments 28, 29, 30).

Pneumococci from which the substance has been extracted in salt solution, those dissolved in bile and by strong solutions of soap (sodium-oleate 1–1,000) no longer yield any symptoms when treated in immune serum and complement or in the latter alone (Table 2, experiments 3, 22, 23).

Boiled virulent pneumococci, non-virulent pneumococci, phenol (0.25 per cent), and chloroform-treated virulent cocci give the substance with more difficulty in immune serum and complement (Table 2, experiments 2, 4, 5, 24). In other words, pneumococci, which we may assume contain an autolytic ferment, produce the anaphylatoxic substance with greatest ease in the mixture with serum. Morphological and tinctorial changes in the pneumococci indicate that the cocci themselves contribute a part at least of this substance, the contrary view of Wasserman and Keysser notwithstanding. In the salt solution extract (see below) they of course are the only source of the poisonous substance.

That the substance in various serum mixtures, which produces anaphylactic intoxication in normal pigs, is an early split product of protein is indicated in Table 3. The proteolytic enzymes in normal untreated serum undoubtedly play a rôle in producing the anaphylatoxin. But the proteolytic enzyme contained in virulent pneumococci also plays a rôle, since, when the latter are added, digestion is carried to the proper point at a lower temperature and (as shown in other experiments) in four hours at 37° C. instead of in 24 hours as required for the serum alone.

The results of a study of the opsonic index to pneumococci and streptococci, just before and shortly after injection of the various mixtures containing pneumococci, show that 10 minutes after death there is no measurable drop in the index in the animals which die in two to four minutes. The serum of those which have severe symptoms but recover show a marked drop in about 15 minutes after the height of the shock, while those in which no symptoms occur show no change in the index. The loss of opsonin is specific for pneumococci. This is of interest especially in connection with the fact that the protein of pneumococci and streptococci are so nearly alike that both sensitize pigs for the other.

TABLE 3.

COMPARISON OF THE EFFECT ON NORMAL GUINEA-PIGS AND THE AMOUNT OF PROTEOLYSIS IN VARIOUS SERUM MIXTURES.

Experiment	Material Injected (4 c.c. in Each Experiment Intravenously)	Formalin Titration	Symptoms in Normal Guinea-Pigs
1	Normal guinea-pig serum	0.4 2.2	No symptoms
2	Normal guinea-pig serum at 37° C. for 24 hrs.	0.45 2.6	Death from typical symptoms in 3 min.
3	Normal guinea-pig serum heated 60° C. for 1 hr.	0.4 2.0	No symptoms
4	Normal guinea-pig serum 15° C. 48 hrs.	0.5 2.3	No symptoms
5	Normal guinea-pig serum at 15° C. 48 hrs., after heating 60° C. 1 hr.	0.2 2.2	No symptoms
6	Normal guinea-pig serum and pneumococci from 30 c.c. broth after treatment in immune serum for ½ hr. at 37° C. at once	0.3 2.2	No symptoms
7	Normal guinea-pig serum and pneumococci from 30 c.c. broth after treatment with immune serum for ½ hr. at 37° C., 150, 48 hrs.	0.6 2.4	Death in 3 min.
8	Normal guinea-pig serum heated and pneumococci from 30 c.c. broth after treatment in immune serum for ½ hr. at 37° C. at once	0.5 2.4	No definite symptoms but very ill
9	Normal guinea-pig serum unheated and pneumococci from 30 c.c. broth treated in ether 37° C. 40 min.	0.6 2.5	Death in 3 min.

The drop in the opsonic index is roughly proportionate to the severity of the symptoms if death is not immediately fatal. It makes no difference whether they are produced in sensitized pigs by the injection of pneumococcus extracts in which autolysis has been largely prevented; in normal pigs with extracts of pneumococci in which autolysis has gone to the proper point; or by the injection of the various serum mixtures, serum broth alone, and serum-broth cultures. In one series of seven experiments the content in hemolytic complement just before and after the injections was tested and a loss was noted in all, even when no symptoms of anaphylaxis occurred. This, however, is greatest when the loss in specific opsonin is most marked.

INTOXICATION WITH EXTRACTS OF PNEUMOCOCCI IN SALT SOLUTION.

As shown in Table 4, symptoms and postmortem findings following the injection of extracts of pneumococci prepared under ether in NaCl solution at 37° C. for the proper length of time, which varies with different strains, are identical with those observed in sensitized pigs following the injection of pneumococcus extracts in which autolysis has been largely prevented. Extracts of virulent pneumococci prepared in the cold, at 37° C. for 24 hours, of boiled virulent pneumococci, and of non-virulent pneumococci at 37° C., both with and without ether or chloroform, do not produce definite symptoms resembling anaphylactic shock in normal pigs. That the toxic substance is an early split product of the pneumococcus protein seems certain because trypsin destroys it (Table 4, Nos. 341 and 342). The formalin titration method[1] of Henriques and Sörensen[2] shows only a slight splitting when symptoms were produced and a greater splitting when trypsin was added and no symptoms were produced. The early toxic effects disappear (Table 4, Nos. 349, 355, 359) on longer residence of the pneumococci at 37° C. in NaCl solution under ether, and finally, no symptoms can be obtained with extracts of non-virulent pneumococci (no autolytic ferment) nor of boiled virulent pneumococci, when the proteolytic enzyme is destroyed.

The amount of protein in the NaCl extracts as compared with serum extracts is exceedingly small—so small that only slight differences are obtained when titrated for proteolysis by the formalin

---

[1] (1) To 10 c.c. of the salt-solution extract or serum mixtures are added 25 c.c. of distilled water and 10 drops of phenolphthalein. This is brought to a permanent pink blush with N/10 KOH. (2) To 5 c.c. of formalin are added 10 c.c. water and the same indicator. This is also brought to a permanent pink. Add 1 to 2 and titrate to a deep red. The first figures given in the tables represent in cubic centimeters the amount of N/10 KOH required to neutralize 10 c.c. extract; the second, the amount required to bring to a deep red after formalin was added.

I am aware of the fact that in the presence of carbonates and phosphates such as occur in serum and broth, the end point of this reaction is somewhat indefinite, but since comparative results only are used, this objection, which might be raised, does not hold. Moreover, precipitating out the carbonates and phosphates gives similar results.

[2] *Ztschr. f. physiol. Chemie*, 1909, 63, p. 27.

TABLE 4.

PRODUCTION OF "ANAPHYLACTIC SHOCK" IN NORMAL GUINEA-PIGS WITH PNEUMOCOCCUS EXTRACTS IN NACL SOLUTION.

No. of Animal	Size of Injection	No. and Preparation of Extract. Place of Injection	Result
341	4 c.c.	Ext. 267*b* prepared in NaCl with chloroform at 37° C. for 24 hrs. Into left heart	Typical symptoms. Death in 10 min. Drop of 3° F. in 10 min. Lungs emphysematous
342	4 c.c.	Ext. 267*a* prepared in NaCl with chloroform and 1–1,000 trypsin. Into left heart. At 37° C. for 24 hrs.	No symptoms
472	7 c.c.	Ext. of virulent pneumococcus 587 in NaCl under ether at 37° C. 48 hrs. Into jugular vein	Typical spasms and dyspnea. Death in 5 min. Drop of 5° F. Lungs emphysematous
472*b*	7 c.c.	Ext. of pneumococcus 575 in NaCl under ether at 37° C. 48 hrs. Into jugular vein	Typical symptoms. Death in 5 min. Drop of 5° F. Lungs emphysematous
479	7 c.c.	Ext. of virulent pneumococcus 587 in NaCl under ether at 37° C. 48 hrs. and heated to 60° C. for ½ hr. Into jugular vein	Some dyspnea, no spasms. Drop of 1° F. in ½ hr. Recovered
475	7 c.c.	*Ibid.*, but after boiling for 10 min. Into jugular vein	No symptoms, drop of 0.5° F. in ½ hr. Remained well
495	7 c.c.	NaCl and ether. Ether removed by passing stream of air through fluid. Into jugular vein	No symptoms. Drop of 0.8° F. in 1 hr.
161	3.5 c.c.	Ext. prepared from virulent pneumococcus in NaCl under ether at 37° C. for 4 days. Into left heart	Profound prostration. Dyspnea 30 sec. after injection, lasting 30 min. Severe chills after 1 hr. Died during night. Autopsy showed ulcer of stomach. Cultures sterile from heart's blood
148	3.5 c.c.	Ext. 162 prepared in NaCl under ether at 37° C. for 4 days. Into left heart	Marked symptoms at once. Died in 11 min. Lungs emphysematous
150	3.5 c.c.	Ext. 164 prepared in NaCl under ether at 37° C. for 48 hrs. Into left heart	Marked symptoms in 4 min. dyspnea, convulsive twitchings, death in 15 min. Moderate pulmonary emphysema
149	3.5 c.c.	Ext. 162 prepared in NaCl under ether at 37° C. for 4 days. Into right ventricle	No definite symptoms, but death during night. Autopsy negative
226	3.5 c.c.	Ext. of virulent pneumococci in NaCl under ether at 37° C. 48 hrs. Into left heart	Severe symptoms at once. Prostration, dyspnea. Died during night. Drop of 3° F. in 1 hr.
222	5 c.c.	Ext. 189*c* prepared in NaCl under ether at 37° C. 48 hrs. Into left heart	Severe symptoms. Labored respiration, spasms, great irritability, but recovered. Temp. dropped 3° F. in 1 hr.
185	6 c.c.	Ext. 171*b* prepared in NaCl under ether at 37° C. for 3 days. Into left heart	Moderately severe symptoms. Leukocytes before injection, 7,200. Leukocytes 1 hr. after injection, 4800

TABLE 4.—*Continued.*

No. of Animal	Size of Injection	No. and Preparation of Extract. Place of Injection	Result
349	4 c.c.	Ext. 269 prepared in NaCl under ether at 37° C. for 24 hrs. Into left heart	Severe symptoms. Drop of 2° F.
355	4 c.c.	Ext. 269 37° C. 48 hrs. Into left heart	No symptoms. Slight rise in temperature
359	4 c.c.	Ext. 269 37° C. 96 hrs. Into left heart	No symptoms of anaphylactic shock. Rise of 2° C. in 1 hr.

NOTE.—Precipitation methods and the formalin-titration method prove that proteolysis was fairly complete where trypsin was added and slight where chloroform only was added (see 341).

method. The extracts which produce primary symptoms show slight splitting; extracts prepared on ice which give no symptoms show no splitting; while those which do not produce symptoms after long residence at 37° C. show the most. The differences, however, are near the border line of experimental error.

The differences in effect on the temperature in normal pigs of extract prepared at 37° C. for varying periods is also shown in Table 4 (Nos. 349, 355, 359); early, when the toxicity is greatest, a drop in temperature occurs, while later the same quantity of extract produces a rise. The toxic substance here, as in serum mixtures, is destroyed largely on heating to 60° C. for $\frac{1}{2}$ hour, and wholly on boiling for 10 minutes (No. 475). The observation that the toxic substance is so easily destroyed by heat is in harmony with the fact that in order to obtain typical symptoms with pneumococcus extracts it is necessary to interrupt autolysis at a certain time.

The extracts of virulent pneumococci which produce symptoms and those which do not because of too long exposure to 37° C., do not produce anaphylaxis in sensitized pigs. In the case of the active extracts this might be explained, with Friedberger and others, on the ground that the proteolytic enzyme contained in the sensitized pig rapidly splits the poison into non-toxic material. In view of the fact that sensitized pigs are relatively immune to small doses of virulent pneumococci, it might be also that the serum of the sensitized pig contains a substance that neutralizes the pneumococcus poison.

Since pneumococcus extracts prepared under ether give anaphylactic symptoms in normal guinea-pigs most regularly, it was necessary to determine whether or not this, in part, is due to the chemical action of the ether on the pneumococci or whether ether only favors the action of the autolytic ferment. Accordingly, experiments were made with two strains of virulent pneumococci. In each case a large quantity of cocci was suspended in NaCl solution thoroughly mixed and divided into two equal parts. Parts *A* were boiled for 10 minutes, then ether added; to parts *B*, ether was added without boiling; all were now placed at 37° C. for 48 hours. At the end of this time the pneumococci were centrifuged, the ether removed, and the toxicity and other properties of the supernatant fluid tested by intravenous injections. The portions boiled before adding ether produced no symptoms, the portions not boiled caused typical symptoms of anaphylactic shock. Formalin titration of the former showed it to be unchanged, while the latter require 0.3 c.c. N/10 KOH to produce pink to phenolphthalein per 10 c.c. of extract after formalin is added.

The amount of coagulable material in my salt solution pneumococcus extracts is so small as compared with the amount of peptone necessary to produce fatal intoxication that its action cannot be due to peptone alone because Kraus and Biedl[1] found that 3 to 4 c.c. of a 10 per cent solution of Witte peptone were necessary to cause fatal intoxication in guinea-pigs weighing 250–300 gms. This is in accord with the work of Spiro and Pick[2] and Papielski,[3] who find that the intoxication following the injection of peptone is not due to peptone, but to other substances present. When viewed from the amount of coagulable substance, phosphotungstic acid producing only a slight turbidity, the material obtained from virulent pneumococci is exceedingly toxic. The results of the formalin titration indicate that this toxic substance is an earlier split product of pneumococci than the peptone stage, and that when this is reached the toxicity disappears.

In order to study more accurately the degree of proteolysis as determined by the formalin-titration method, in relation to the symptoms produced in normal pigs, further experiments (Table 5) were made with serum and serum-broth cultures. Here protein splitting is more marked because of a larger amount present than the extract in salt solution. The ability of the serum-broth mixtures to produce intoxication with symptoms like those of anaphylaxis bears a close relation to the degree of proteolysis. It makes no difference, however, whether the splitting is brought about by the complement in the serum broth alone (Table 5, experiments 1, 2, 8, and 9), by longer residence at 37° C., or earlier by complement, aided by the proteolytic enzyme contained in the virulent pneumococci (experiments 3, 4, 5, and 6), or finally by the latter alone on longer exposure to 37° C. in broth alone (experiments 10 and 11).

---

[1] *Wien. klin. Wchnschr.*, 1909, 22, p. 363.

[2] *Ztschr. f. physiol. Chemie*, 1900, 31, p. 235.

[3] *Pflüger's Arch.*, 1909, 126, p. 483.

TABLE 5.

Effect of Serum Broth and Broth on Guinea-Pigs in Relation to Intoxication Produced by Pneumococci.

Experiment	Normal Guinea-Pig Serum One Part, Meat Dextrose 2 per cent, Broth Nine Parts	Formalin Titration	Symptoms in Normal Guinea-Pigs. 4.5 c.c. Intravenously
1......	Serum broth. Im.	0.8 3.4	No symptoms
2......	Serum broth at 37° C. 24 hrs.	1.0 4.6	No symptoms
3......	Serum broth and pneumococci from 30 c.c. broth culture at 37° C. for 4 hrs.	2.9 5.0	No symptoms
4......	Serum broth and pneumococci from 30 c.c. broth culture at 37° C. for 24 hrs.	2.6 5.8	Death in 4 min.
5......	Serum broth and pneumococci from 30 c.c. broth culture at 37° C. for 48 hrs.	3.0 5.8	Very severe symptoms
6......	Serum broth and pneumococci from 30 c.c. broth culture at 15° C. for 48 hrs.	3.0 5.4	Moderate symptoms
7......	Serum broth and pneumococci from 30 c.c. broth culture at once	1.5 4.4	No symptoms
8......	Serum broth at 37° C. 48 hrs.	1.1 4.4	Slight symptoms
9......	Serum broth at 37° C. 96 hrs.	1.6 6.2	Profound symptoms, nearly died
10......	Meat dextrose 2 per cent broth at 37° C. for 96 hrs.	0.8 2.45	No symptoms
11......	Meat dextrose 2 per cent broth after highly virulent pneumoccci are grown in it for 96 hrs.	3.2 5.2	Moderately severe symptoms

SUMMARY.

Anaphylaxis to pneumococcus protein does not differ materially from that to other proteins.

When pneumococcus extracts (antigen), in which autolysis has not gone too far, are injected into sensitized animals, there occurs a rapid splitting into a toxic substance.

In the sensitized guinea-pig, autolyzed and heat-killed cocci as well as living non-virulent and virulent pneumococci are dissolved more rapidly than in the normal animal. The relatively slight yet definite immunity of sensitized guinea-pigs to virulent pneumococci is probably largely due to this cause.

It is possible to obtain from virulent pneumococci by autolysis in NaCl solution a substance which produces symptoms, fatal and otherwise, wholly like those of anaphylaxis in normal guinea-pigs, just as from mixtures of pneumococci and serum. This substance is probably an early split product of pneumococcus protein and in its action is identical with that obtained with the mixture of pneumococci and serum.

Autolysis of normal serum, digestion in serum-broth mixtures, and digestion of pneumococcus protein in serum-broth and broth cultures yield a substance or substances with the same effect. In all these cases, it seems to concern a proteolytic action. When this is comparatively slow a higher temperature for a longer time yields the same result as the more active process at a lower temperature.

Pneumococcus anaphylatoxin depresses the leukocytic mechanism. Autolyzed pneumococci, from which this substance has been removed, on the contrary, have the power of stimulating the leukocytic mechanism in sensitized guinea-pigs. Further digestion in serum or in salt solution under ether destroys this property also. This affords the best explanation we have (1) of the development of leukopenia in overwhelming experimental pneumococcus infections, and in lobar pneumonia in man, when digestion of pneumococci, we may assume, is imperfect and there is liberated a large amount of anaphylatoxin; (2) of the high leukocytosis in severe but more favorable infections where digestion is carried rapidly to a farther point, and there is liberated a relatively large amount of the latter substance which stimulates the leukocytic mechanism, and (3) of the slight leukocytosis in the mild infections where digestion of pneumococci is still more rapid, and where both anaphylatoxin and the leukocytogenic substance are rapidly digested into relatively harmless products.

The action of pneumococcus anaphylatoxin is identical with other protein anaphylatoxins, but when considered from the standpoint of the amount of precipitable substance contained in pneumococcus extracts, pneumococci yield a very much larger proportion of this cleavage product than does serum or egg white during digestion with serum.

The fact that virulent pneumococci have within themselves a proteolytic enzyme which splits their protein into a highly toxic substance is strong indication that certain strains of pneumococci may cause infection forthwith without first rendering the host allergic. This is quite in keeping with the fact that in pneumococcus infections an incubation period is not an invariable rule. On the other hand, in certain instances, a previous sensitization before severe symptoms set in probably occurs. This might well be the case in lobar pneumonia when the chill occurs a week or 10 days after the patient contracted a severe cold or bronchitis. The distribution by lobes in typical cases may be related to the bronchial spasm which this toxic substance produces. The early dyspnea and increased respiration before consolidation is demonstrable is in keeping with this idea.

The fact that the allergic state or hypersensitiveness in guinea-pigs to pneumococcus extracts disappears after repeated inoculations of dead pneumococci or pneumococcus extracts with severe anaphylactic shock, and after recovery from experimental pneumococcus infections, suggests the possibility that the crisis in lobar pneumonia, for example, is a reaction against anaphylatoxin either in the nature of the development of an anti-anaphylatoxin, or what seems more likely, by the development of enzymes, to a point where rapid splitting of anaphylatoxin into non-toxic products occurs.